

## Research Article

# Influences of Farming Practices on Soil Properties and the 2-Acetyl-1-pyrroline Content of Khao Dawk Mali 105 Rice Grains

Kawiporn Chinachanta<sup>1,2</sup>, Laetitia Herrmann<sup>3</sup>, Didier Lesueur<sup>3,4,5,6</sup>,  
Sakda Jongkaewwattana<sup>2</sup>, Choochad Santasup<sup>2</sup> and Arawan Shutsrirung<sup>2</sup>

<sup>1</sup>Doctor of Philosophy Program in Environmental Soil Science, Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>2</sup>Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

<sup>3</sup>Alliance of Bioversity International and Centre International d'Agriculture Tropicale (CIAT), Asia hub, Common Microbial Biotechnology Platform (CMBP), Hanoi, Vietnam

<sup>4</sup>Centre de Cooperation Internationale en Recherche Agronomique pour le Developpement (CIRAD), UMR Eco&Sols, Hanoi, Vietnam

<sup>5</sup>Eco&Sols, University of Montpellier (UMR), CIRAD, Institut National de la Recherche Agronomique (INRAE), Institut de Recherche pour le Developpement (IRD), Montpellier SupAgro, 34060 Montpellier, France

<sup>6</sup>School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia

Correspondence should be addressed to Arawan Shutsrirung; [arawan.s@cmu.ac.th](mailto:arawan.s@cmu.ac.th)

Received 27 May 2020; Revised 12 November 2020; Accepted 29 November 2020; Published 21 December 2020

Academic Editor: Maman Turjaman

Copyright © 2020 Kawiporn Chinachanta et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Khao Dawk Mali 105 (KDML105) is a premium fragrant rice variety and is widely grown in Thung Kula Rong Hai (TKR), northeast Thailand. In the present study, the influence of organic and conventional rice farming (ORF and CRF, respectively) in TKR farmers' paddy fields on soil properties and their relationship with 2-acetyl-1-pyrroline (2AP) in KDML105 rice grains were investigated. The results indicated that the ORF system had a strong positive effect on major soil quality indicators and the 2AP content in the rice grains. The soil organic matter (SOM) was approximately twice as much in the ORF than in the CRF system, thus leading to much higher total nitrogen (TN), humic acid (HA), and microbial populations in the ORF system. The higher SOM in the ORF system not only enhanced the soil quality indicators but also contributed to approximately 3.5 times higher 2AP than in the CRF system. Principle component analysis indicated a close correlation among SOM, TN, HA, and microbial population under the ORF system; these variables exhibited strong correlations with the 2AP contents in KDML105 rice grains.

## 1. Introduction

Thai aromatic rice, especially the Khao Dawk Mali 105 (KDML105) cultivar, is the most popular rice type globally, owing to its high cooking quality and unique aroma. The KDML105 variety was declared the world's best rice at the World Rice Conference held in Macau in 2017. The price of KDML105 in the international rice market is almost double that of other rice cultivars. The volatile aromatic compounds of KDML105 have been studied extensively by many researchers.

The 2-acetyl-1-pyrroline (2AP) aroma compound was first determined in 1983; since then, it has been considered as the most significant aroma compound in rice [1], including in the KDML105 variety. There are many factors that affect the strength of rice aroma, such as soil properties, genetic conditions, light intensity, and climatic conditions [2–4]. Different fragrant rice varieties grown around the world have different aroma levels. Among these varieties, the KDML105 (Jasmine rice), Italian, and Basmati rice varieties contain quite high 2AP levels. In addition to the rice genotype, certain growing

environment characteristics such as soil salinity, soil nutrients, drought conditions, storage time and temperature, planting density, and harvesting time exert considerable effects on aroma strength and quality [4, 5]. Low density and early harvesting have been shown to improve the aroma content and other seed qualities [6, 7].

Although the KDML105 rice variety is widely grown throughout Thailand, at present, the most premium quality of the variety in terms of unique aroma and 2AP quantity is produced in the 'Thung Kula Rong Hai (TKR) area. TKR is located in the centre of northeast Thailand and covers a wide plain area of 2.1 million rai that extends across five provinces, namely, Roi Et, Maha Sarakham, Surin, Yasothon, and Srisaket. Approximately 46% of the area belongs to Roi Et Province. The TKR region is underlain with tremendous rock salt layers of the Maha Sarakham formation (mainly halite (NaCl)), which cause major problems for agricultural activities. In addition, the soils are sandy, acidic, and infertile [8]. Soil quality improvement has been carried out in TKR by the Land Development Department since 1981. Currently, the KDML105 rice variety produced in the TKR region is well known for its premium quality, unique taste, and distinct smell. A number of studies have been undertaken for more than a decade to understand the factors that affect the unique taste and aroma of the KDML105 rice variety; however, to date, scientists have not yet reached a definitive conclusion. It has been assumed that the KDML105 rice quality is highly influenced by the photoperiod, wet and dry conditions, climate, and soil nutrients of TKR. The combination of stress during rice cultivation in TKR may stimulate the rice to respond by producing proline substances, which are the precursors of the aromatic substance (2AP) of KDML105. In addition, the concentration of 2AP is influenced by interactions between the rice genotype and environmental factors, such as soil fertility and abiotic stress. Under high soil salinity, KDML105 cells accumulate  $\text{Na}^+$ , leading to proline and 2AP increases in the rice grains. Osmoprotectant proline has been found to be the precursor and the nitrogen (N) source of 2AP in KDML105 [9]. High total soil N increases the 2AP content in grains [10]. Some micronutrients such as Mn, Si, and Zn also appear to be related to 2AP levels in aromatic rice [11].

Recently, the aroma quality in the KDML105 and other scented rice varieties has undergone a gradual degradation. The progressive reduction in the 2AP levels of KDML105 may be due to soil quality degradation caused by high agrochemical applications in conventional farming. For these reasons, the objectives of the present study was (1) to determine the soil properties of the farmers' paddy field in the TKR areas influenced by organic rice farming (ORF) and conventional rice farming (CRF), (2) to analyse the 2AP content in KDML rice grains collected from ORF and CRF, and (3) to analyse the interrelationship between farming practice, soil property, and the 2AP content in KDML 105 rice grains.

## 2. Materials and Methods

**2.1. Soil and Rice Grain Sampling.** Eighteen farmers' rain-fed paddy fields (nine organic and nine conventional KDML105

rice fields) in TKR and neighbouring areas of Surin and Yasothon provinces, well-known rice growing areas, were selected for soil and rice grain sampling. The ORF paddy fields have been registered as organic for around 5 years. The rice in CRF paddy fields has been cultivated for 15 years. The ORF was maintained under the Thai Organic Agricultural Standard (TAS 9000–2009) with the application of composted manure ( $625 \text{ kg ha}^{-1}$ ) and green manures. The CRF was practiced under the Thai Agricultural Standard (TAS 4400–2009) with the application of chemical fertilizers: 46-0-0 ( $93.8 \text{ kg ha}^{-1}$ ) and 16-16-8 ( $156.2 \text{ kg ha}^{-1}$ ).

The samples used in this study were collected from the farmers' field during the dry season before the rice harvest (November 2018). Rhizosphere soil samples were randomly collected (0–15 cm deep), in 10 spots per composite soil sample. Each sample was divided into two parts. One part was preserved in field-moist condition and was used for microbial analysis, and the other was air-dried for physicochemical analysis. Rice grain samples were also collected from the same 18 sites. The 18 sites extended over five provinces (Roi Et, Maha Sarakham, Surin, Yasothon, and Srisaket) (Figure 1).

**2.2. Soil Property Analysis.** The soil texture, pH, and EC were analysed by the standard method [12, 13]. The cation-exchange capacity (CEC) was determined using the leaching method [14, 15]. Humic acids (HA) were determined using the method of Ahmed et al. [16] and Palanivell et al. [17]. The soil organic matter (SOM) was determined using the Walkley–Black method [18]. The total N (TN) content of the soils was determined using the modified micro-Kjeldahl digestion method [17]. Phosphorus (P) and sulphate (S) were extracted by Bray II and  $\text{Ca}(\text{H}_2\text{PO}_4)_2$  in 2N HOAc, respectively, and determined by using a spectrophotometer [19, 20]. The exchangeable bases, i.e., potassium (K), calcium (Ca), and magnesium (Mg), were extracted with 1N ammonium acetate ( $\text{NH}_4\text{OAc}$ ) and determined by atomic absorption spectrophotometry [21].

**2.3. Determination of the Microbial Population.** Exactly 10 g of each soil sample was mixed into 95 mL of sterile distilled water and shaken (120 rpm) for 30 min. Serial dilutions were prepared, and 0.1 mL aliquots ( $10^3$ – $10^5$ ) were spread on agar media plates. Egg albumin and rose bengal agar medium [22] plates were used to measure the total bacterial and actinomycetal populations and fungal populations, respectively. The total counts were determined after 3–5 days of incubation.

**2.4. Determination of 2AP Content in KDML 105 Rice Grains.** The rice grain samples were dried in an oven at  $60^\circ\text{C}$  for 3 days until the moisture content was reduced to 14%. The rice grain samples were, then, dehusked by hand to yield uncooked brown rice seeds before being sent to the Chemistry Laboratory, Faculty of Science, Chiang Mai University, Thailand, for the determination of the 2AP content by the method of Wongpornchai et al. [23].

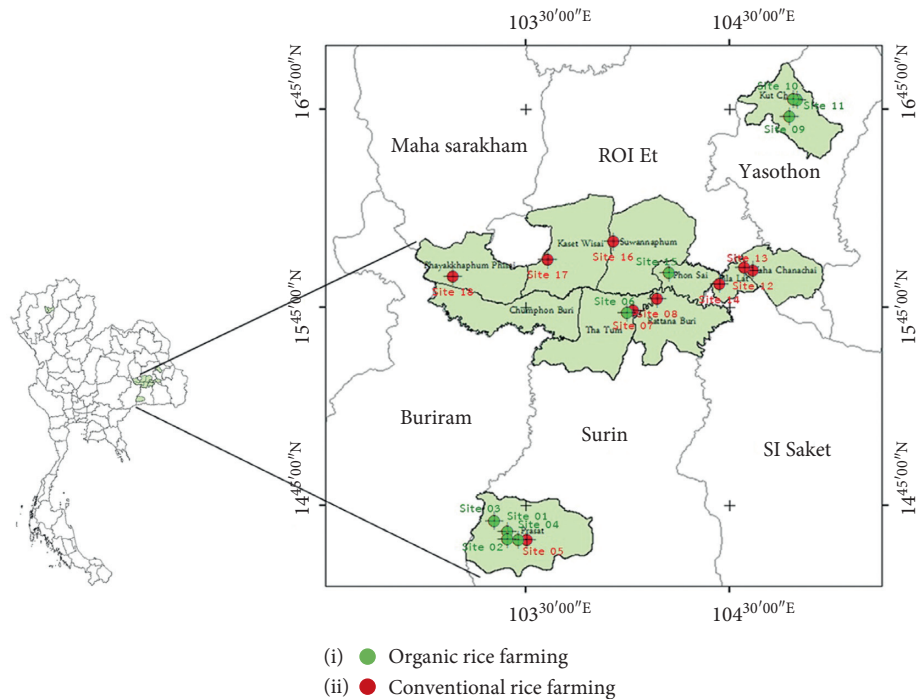


FIGURE 1: Eighteen sampling sites in the Tung Kula Rong Hai region and neighbouring areas of Surin and Yasothon provinces.

**2.5. Statistical Analysis.** The data were compared statistically by analysis of variance (ANOVA) with Duncan's multiple range test at the 0.05 probability level in Statistix 8.0. Arithmetic means were calculated for each of the three replicates separately. The obtained data were analysed statistically (correlation) using the SPSS Statistics for Mac OS X, version 20 (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed to evaluate the relationships among the different cultivation systems, soil properties, and the 2AP content. Soil parameters including pH, EC, SOM, HA, CEC, TN, P, K, Ca, Mg, and S and the microbial population, as well as the sand, silt, and clay percentages, were introduced as variables in the PCA using R 1.2.1335 [24].

### 3. Results and Discussion

Jasmine rice var. KDML105 is officially one of the best aromatic rice varieties because of its unique fragrance. We hypothesized that farming practices and soil properties might affect the aromatic quality of rice grains. Therefore, in the present study, the influence of farming practices in TKR paddy fields on soil properties and their intercorrelation with 2AP were investigated.

**3.1. Soil Properties Affected by Farming Practice.** Sandy soils are widespread in the TKR region. Some areas have sandstone-derived soils, while in other, soils are severely affected by salt [25]. In the present study, most of the soils in the study area (72%) had a sandy texture (loamy sand/sandy loam) (Figure 2). The soil texture of the rest of the locations ranged from loam to silt loam. The sand, silt, and clay

percentages under the same soil texture were similar in the two farming practices (ORF and CRF) (Figure 2). In general, soil texture is a fixed characteristic and cannot be changed unless a significant volume of these components is added or subtracted. The results of this study confirmed that the different farming practices did not change the soil texture. However, soil aggregates bearing on other soil properties may be highly affected by the farming practices.

On the average, a slight difference in the pH of paddy soils was observed between the ORF (4.87) and CRF (5.37) systems (Figures 3(a) and 3(b); Table 1). Several studies have shown that soil pH is slightly but not significantly lower in organic systems compared to conventional systems (on similar soils) [26]. In general, flooding in acidic paddy soils leads to strongly reducing conditions in the topsoil and pH increase to near neutrality [27]; however, the soil pH decreases after draining [28]. In the present study, the rhizosphere soils were collected at harvest time when they were almost dry, and then, they were air-dried before analysis. Therefore, the soil pH of ORF and CRF under this study was influenced by farming practice rather than submerged conditions.

Most of the EC values of the ORF system ( $0.54\text{--}2.54\text{ dS m}^{-1}$ ) were significantly higher than those of the CRF system ( $0.18\text{--}1.54\text{ dS m}^{-1}$ ) (Figures 3(c) and 3(d)). The average EC value of the ORF system ( $1.25\text{ dS m}^{-1}$ ) was much higher than that of the CRF system ( $0.53\text{ dS m}^{-1}$ ) (Table 1). In the present study, the higher EC found in the ORF than in the CRF system might be affected by organic inputs in the ORF system. Our findings are in good agreement with previous works in that organic practices including the addition of green manures, organic matter, and compost to soils markedly increased the EC values [29–32].

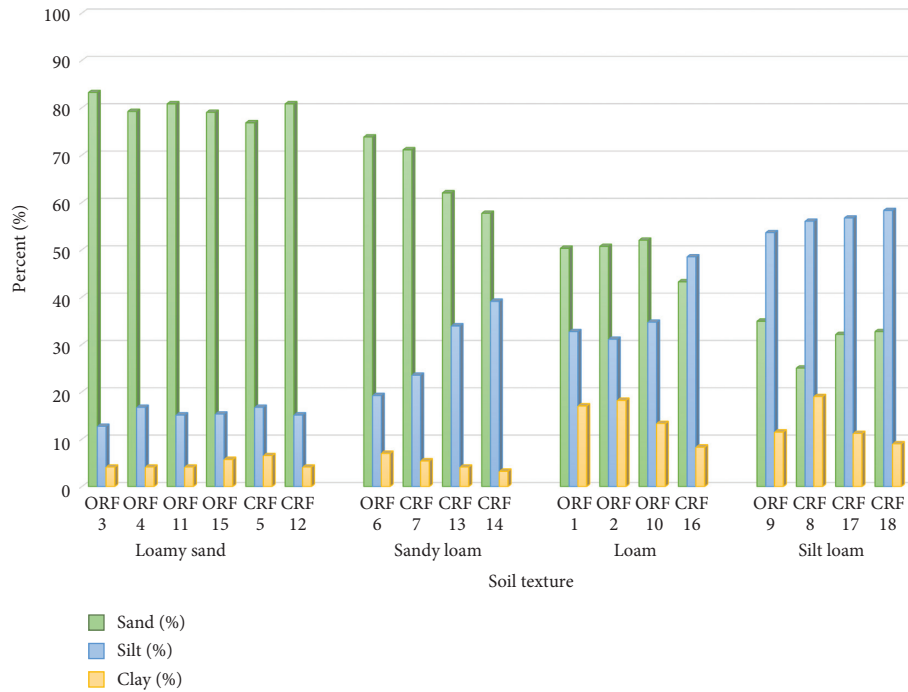


FIGURE 2: The proportion of sand, silt, and clay particles in the soil of organic and conventional rice farming (ORF and CRF, respectively) sites for each soil textural class.

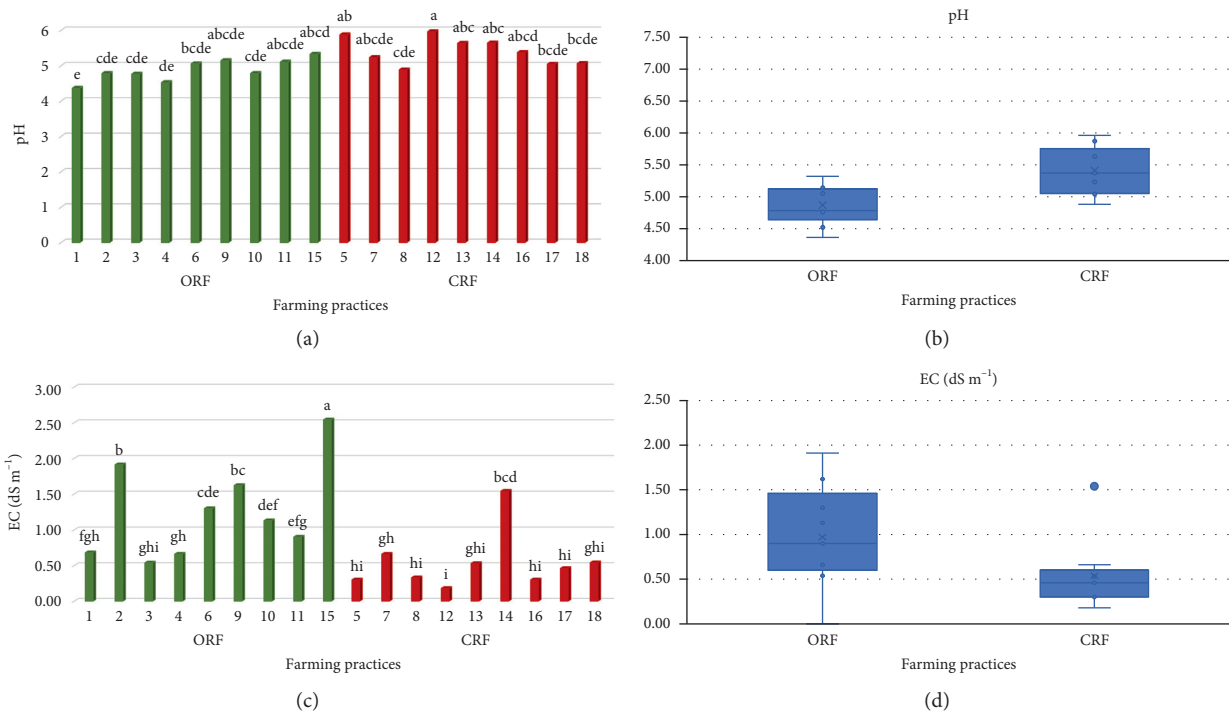


FIGURE 3: The soil pH value (a, b) and electrical conductivity (c, d) of the soils in organic and conventional farming systems (ORF and CRF, respectively). The error bars represent the standard deviation of measurements for nine soil samples.

The amount of SOM in the soil samples was very low to quite high, ranging from 0.30% to 2.41%, and the average of ORF-SOM (1.41%) was higher than the average of CRF-SOM (0.66%) (Figure 4(a) and 4(b); Table 1). The SOM of rain-fed sandy loam in the TRK region is naturally quite low

(1.05%) [33]. The SOM content is influenced by various factors such as soil texture and farming practices [34, 35]. The SOM of clay loam and sandy loam in the TKR region is approximately 1.04% and 0.62%, respectively [36], indicating the influence of soil texture on SOM. However, with

TABLE 1: Statistical summary of the physicochemical properties and microbial population of the soil for organic and conventional rice farming systems.

Variable	Unit	Organic rice farming				Conventional rice farming			
		Minimum	Maximum	Mean	S.D.	Minimum	Maximum	Mean	S.D.
pH		4.36	5.32	4.87	0.31	4.88	5.96	5.41	0.39
EC	dS m <sup>-1</sup>	0.54	2.54	1.25	0.67	0.18	1.54	0.54	0.40
SOM	%	0.84	2.41	1.41	0.55	0.30	1.03	0.66	0.23
Humic acid	%	1.05	1.78	1.29	0.25	0.24	1.47	0.74	0.40
CEC	cmol <sub>c</sub> kg <sup>-1</sup>	1.98	7.66	4.22	2.25	1.19	5.15	3.01	1.36
Total N	%	0.04	0.12	0.07	0.03	0.02	0.05	0.03	0.01
Avail. P	mg kg <sup>-1</sup>	2.44	37.53	13.68	13.92	2.38	139.16	30.12	45.53
Exch. K	mg kg <sup>-1</sup>	34.08	131.91	73.70	35.57	9.01	159.49	67.28	49.25
Exch. Ca	mg kg <sup>-1</sup>	101.72	1307.35	377.50	376.49	59.00	651.96	262.57	194.36
Exch. Mg	mg kg <sup>-1</sup>	9.46	197.46	42.51	59.53	2.66	31.16	17.79	11.13
Extr. S	mg kg <sup>-1</sup>	9.10	222.99	38.96	69.41	2.53	24.78	10.40	6.11
Sand	%	34.90	83.20	64.88	17.86	25.00	80.80	53.49	20.91
Silt	%	12.70	53.60	25.68	13.46	15.10	58.30	38.64	17.32
Clay	%	4.10	18.20	9.44	5.68	3.20	19.00	7.87	4.93
Bacteria	( $\times 10^5$ ) cfu g <sup>-1</sup>	80.51	620.60	277.12	186.99	10.12	50.72	22.61	15.78
Actinomycetes	( $\times 10^5$ ) cfu g <sup>-1</sup>	2.08	23.70	12.98	8.84	0.02	1.56	0.91	0.54
Fungi	( $\times 10^5$ ) cfu g <sup>-1</sup>	0.42	1.78	1.00	0.37	0.11	0.29	0.22	0.06

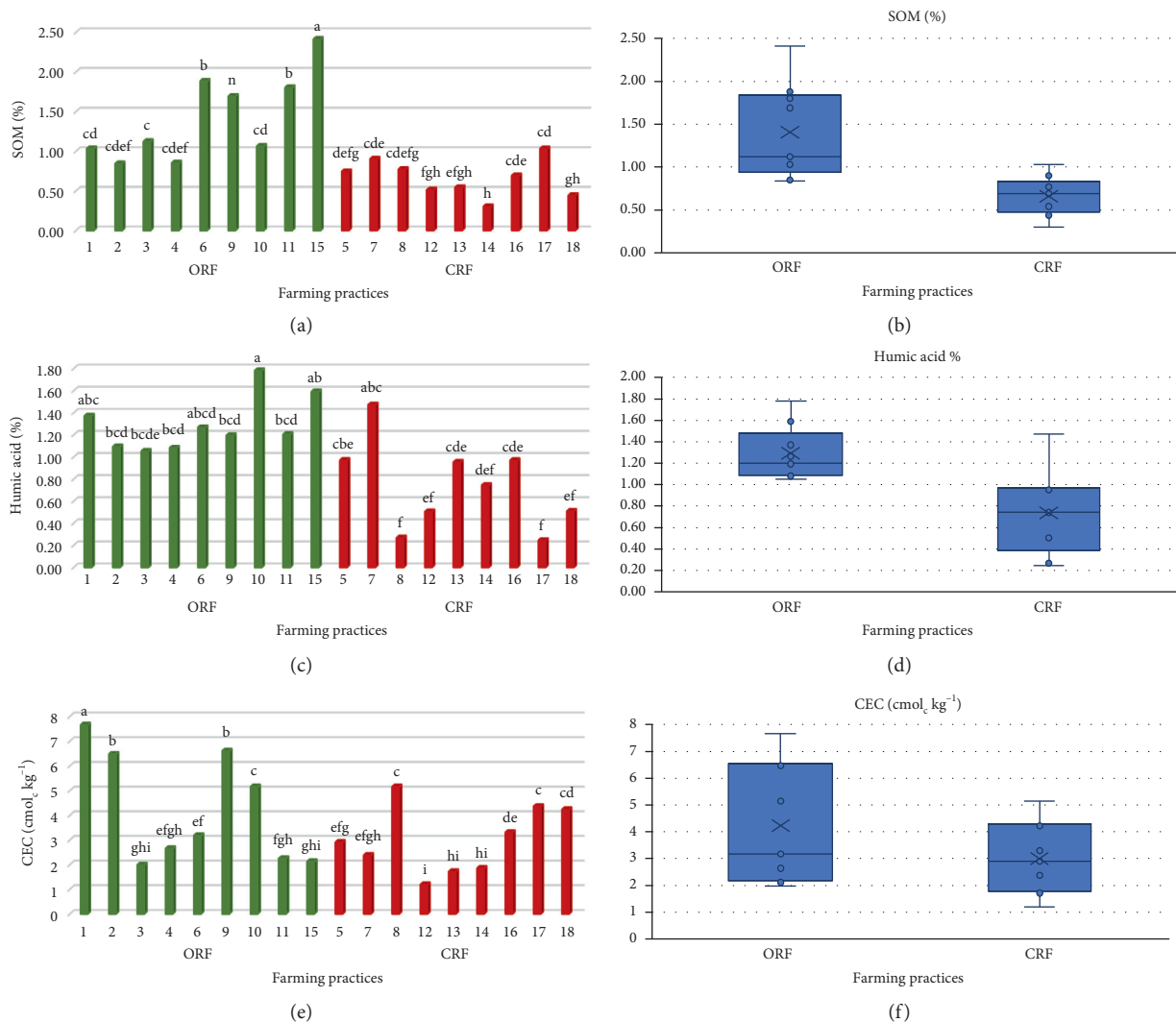


FIGURE 4: Soil organic matter (SOM) (a, b), humic acid (c, d), and cation-exchange capacity (CEC) (e, f) of the soils in organic and conventional farming (ORF and CRF, respectively). The error bars represent the standard deviation of measurements for nine soil samples.



the organic fertilizer application in the ORF system in this study, the SOM value in sandy loam and loamy sand under this system could be as high as 1.9% and 2.41%, respectively (Figures 2 and 4(a)). The results demonstrated the higher impact of organic practices on SOM than the soil texture. Under submerged conditions in acidic soils, the pH increase is faster in soils with high SOM than in those with low SOM [27]; thus, organic practice would benefit in terms of enhancing soil buffering capacity in the paddy field.

The application of various organic matter types into soils increased the amount of humic acid (HA) [37]. HA derived from SOM decomposition is an important fraction in the formation and stability of water-stable aggregates, thus improving the movement of water and air in the soil. In the present study, the application of organic matter in the ORF system appeared to increase the SOM and HA content irrespective of the soil texture (Figures 2 and 4). The average HA values in the soils of the ORF system (1.29%) were much higher than those of the CRF system (0.74%) (Table 1). HA enhances enzyme activities involved in photosynthetic metabolism in maize leaves [38] and improves rice yields by 10–20% [39]. In this study, therefore, the high HA in the ORF system had a high potential to increase the physico-chemical and biological properties of the soil of TKR paddy fields, thus also increasing rice yields.

The cation-exchange capacity (CEC) of soils is mainly due to SOM and clay minerals. The TKR soil is sandy in nature (low SOM and clay contents); therefore, the CEC value of natural TKR soil is quite low (2.31 to 7.51  $\text{cmol}_c \text{kg}^{-1}$ ) [33, 40]. The CEC values of the TKR soils in this study are in good agreement with earlier reports with values ranging from 1.19 to 7.66  $\text{cmol}_c \text{kg}^{-1}$  (Figures 4(e) and 4(f)). The average CEC values were 4.22 and 3.01 ( $\text{cmol}_c \text{kg}^{-1}$ ) for the ORF and CRF systems, respectively (Table 1). On average, the CEC of the ORF system under all soil textural classes was higher than that of the CRF system, particularly under the loam and silt loam class. The results indicated that the CEC of soils in this study was mainly due to SOM and, to a lesser extent, clay mineral (Figure 5). Studies have indicated that the contribution of organic matter to the total CEC of a soil is usually substantial and is often considerably greater than that of clay minerals [41].

Although more than 80% of the TKR soil is considered as poorly fertile soil [40], our results showed that the ORF practice could improve several nutrients level, particularly the total N (TN), as compared to the CRF practice (Figure 6(a)). The organic fertilizer application in the ORF system increased the TN amount (0.037%) by approximately twice as much as that of the CRF system (0.017%) (Figure 6(a)). An earlier report also indicated that the application of compost in a maize-wheat cropping system increased the initial TN value up to 78–93% [42]. Beside TN, the average values of exchangeable K, Ca, and Mg were higher in the ORF system (73.7  $\text{mg kg}^{-1}$ , 377.5  $\text{mg kg}^{-1}$ , and 42.51  $\text{mg kg}^{-1}$ , respectively) than in the CRF system (67.3  $\text{mg kg}^{-1}$ , 262.6  $\text{mg kg}^{-1}$ , and 17.8  $\text{mg kg}^{-1}$ , respectively) (Table 1; Figures 6(c)–6(e)). In contrast, the *p* values of the CRF system were higher than those of the ORF system, with mean values of 30.12  $\text{mg kg}^{-1}$  and 13.68  $\text{mg kg}^{-1}$ ,

respectively (Table 1). In the present study, it appeared that the ORF practice had a positive impact on the Ca, Mg, and S amounts, but not on the K amount. The same trend was found with extractable S, with the average value of S in the ORF system (38.96  $\text{mg kg}^{-1}$ ) being obviously higher than that of the CRF system (10.4  $\text{mg kg}^{-1}$ ) (Figure 6(f)).

Soil microbes and their functions, particularly SOM decomposition, humification, and nutrient transformation, are key factors for the sustainability of soil quality, agricultural systems, and ecosystem services. The results of the present study showed that the SOM, HA, and TN values were obviously higher in the ORF system (Figures 4 and 6). As a result of high SOM, the bacterial, actinomycetal, and fungal populations in the rhizosphere soils examined in this study were much higher in the ORF than in the CRF system (Figure 7). High bacterial numbers were detected in the ORF system, with values ranging from 8.51 to 62.6 ( $\times 10^6 \text{ cfu g}^{-1}$ ). Much lower bacterial numbers were detected in the CRF system, with values ranging from 1.17 to 5.72 ( $\times 10^6 \text{ cfu g}^{-1}$ ) (Figure 7(a)). The actinomycetal population ranged from 2.08 to 23.7 ( $\times 10^5 \text{ cfu g}^{-1}$ ) and 0.02 to 1.56 ( $\times 10^5 \text{ cfu g}^{-1}$ ) in the ORF and CRF systems, respectively (Figure 7(b)). A similar trend was observed in the fungal populations; these ranged from 4.19 to 17.8 ( $\times 10^4 \text{ cfu g}^{-1}$ ) and 1.12 to 2.88 ( $\times 10^4 \text{ cfu g}^{-1}$ ) in ORF and CRF, respectively (Figure 7(c)). The results were in good agreement with an earlier study that higher microbial population and activity were recorded in soils under the ORF than under the CRF system [43].

Microbes in the rhizosphere can enhance plant growth and immunity by providing secondary metabolites (SMs). Under stress conditions in rice-growing areas of TKR, high rhizosphere microorganisms in the ORF system might stimulate the production of SMs, particularly 2AP in rice, leading to higher stress tolerance and aroma level in the grains.

**3.2. Correlation between Soil Properties and 2AP in Rice Grains.** 2AP has proven to be a potent N-containing aroma compound in fragrant rice varieties. 2AP is a volatile alkaloid substance in rice that normally accumulates in response to environmental stress [44]. In the TKR region, the unique and high 2AP aroma of KDML105 may be due to various stress such as the natural low fertility of sandy soil, the high salt content, and the water shortage during the rain-fed rice season [40]. Therefore, in this study, we determined the 2AP concentration in mature rice grains and analysed the relationship between 2AP and the soil properties of the ORF and CRF systems.

**3.2.1. 2AP Affected by Farming Practice.** Several studies have shown that organic inputs not only improve rice soil but also enhance rice quality including 2AP compared to chemical fertilizer applications [45, 46]. The 2AP level in rice grains is widely used as one of the high-quality indicators of aromatic rice. In the present study, the 2AP levels of all ORF sites were higher than those of the CRF sites (Figure 8(a)). The 2AP contents of TKR rice grains obtained from the ORF system (5.52–13.69  $\text{mg kg}^{-1}$ ) were significantly ( $p < 0.05$ ) higher

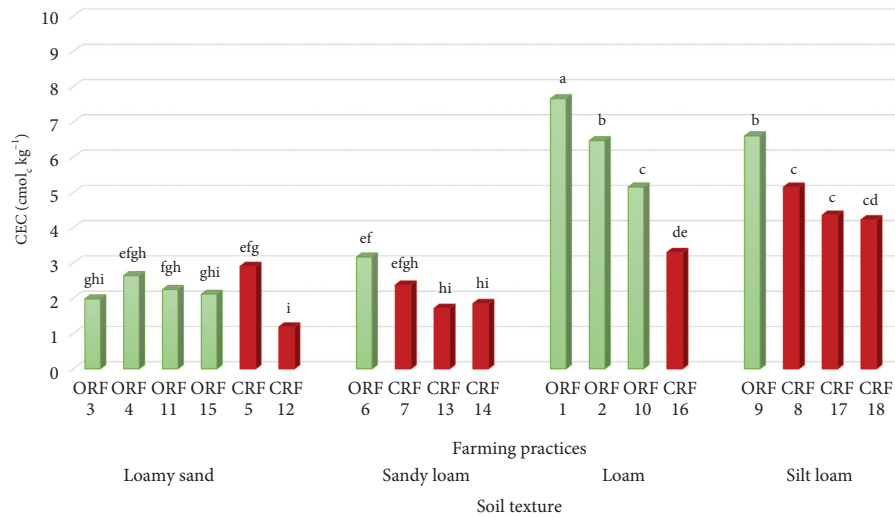


FIGURE 5: Cation-exchange capacity (CEC) as influenced by soil texture and farming practices. Note: ORF=organic rice farming; CRF=conventional rice farming.

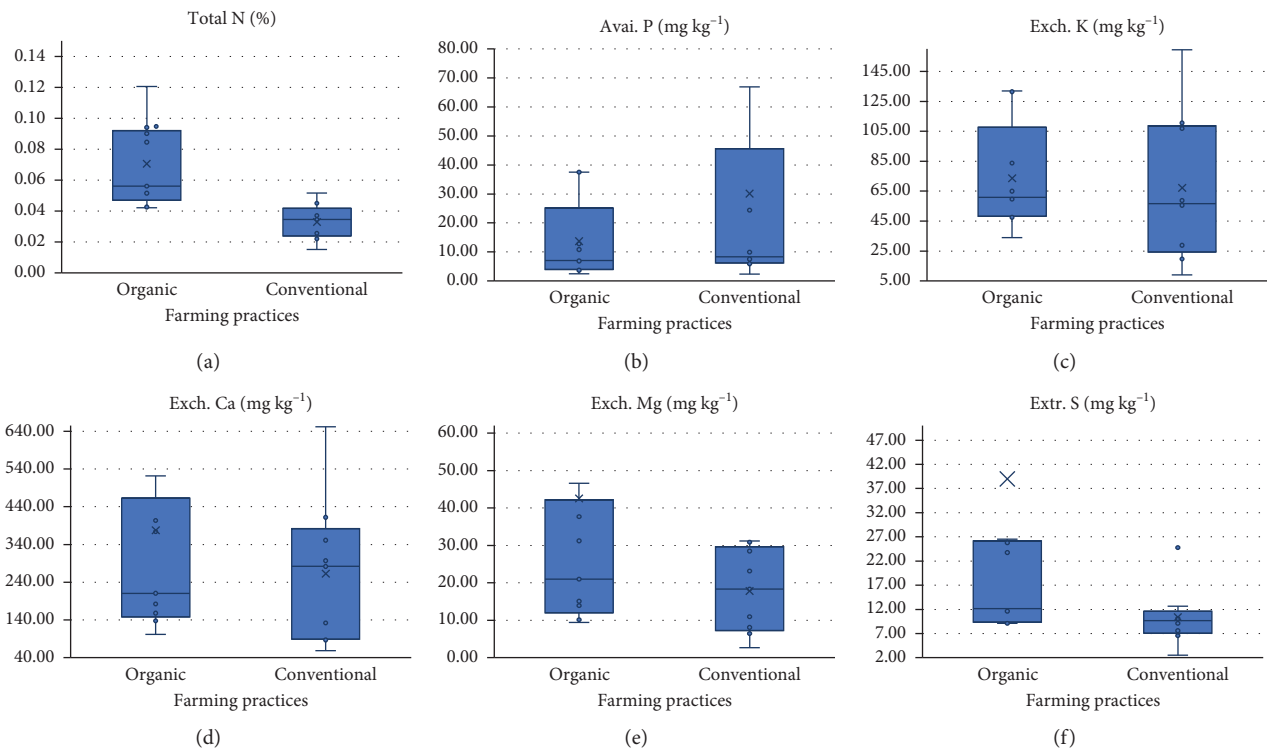


FIGURE 6: Soil mineral concentration (a–f) in soils collected from organic and conventional farming (ORF and CRF, respectively) systems. The error bars represent the standard deviation of measurements for nine soil samples.

than those obtained from the CRF system ( $0.49\text{--}5.60\text{ mg kg}^{-1}$ ) (Figures 8(a) and 8(b)). Poomipan et al. [47] reported that the 2AP in rice grains obtained after organic fertilizer application ( $1.77\text{ mg kg}^{-1}$ ) was higher than that obtained after chemical fertilizer application ( $1.46\text{ mg kg}^{-1}$ ). The results obtained in this study confirm that the ORF system provided much higher 2AP in the rice grains over the CRF system.

**3.2.2. 2AP Affected by Soil Chemical Properties.** Several studies have shown that soil texture may influence the 2AP level in mature rice grains. An evaluation of 67 soil samples in the TKR region revealed that the level of 2AP in rice grains grown in sandy soils is higher than that in those grown in loamy soils, followed by clayey soils [40]. A higher 2AP content was found in rice grains grown in sandy soils ( $1.90\text{--}3.00\text{ mg kg}^{-1}$ ) than in those grown in clayey soils

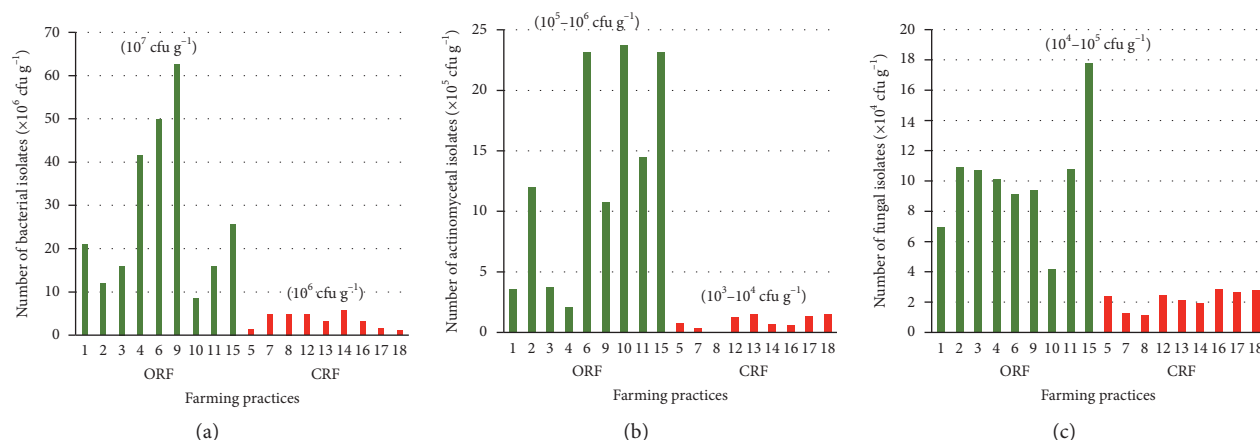


FIGURE 7: Bacterial (a), actinomycetal (b), and fungal (c) populations in the rice rhizosphere under different cultivation systems. The error bars represent the standard deviation of measurements for nine soil samples. Note: ORF = organic rice farming; CRF = conventional rice farming.

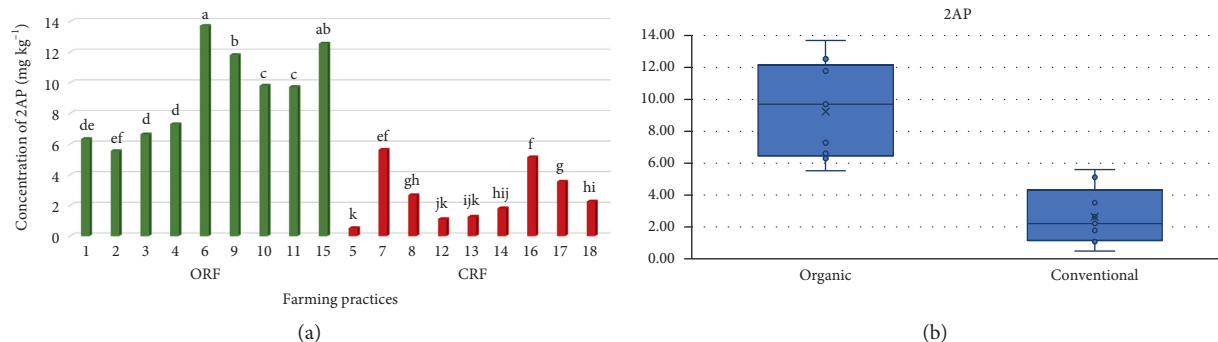


FIGURE 8: The 2-acetyl-1-pyrroline (2AP) contents of Khao Dawk Mali 105 (KDML105) grains grown under different cultivation systems. The error bars represent the standard deviation of measurements for nine soil samples. Note: ORF = organic rice farming; CRF = conventional rice farming.

( $1.00\text{--}1.50 \text{ mg kg}^{-1}$ ) [48]. However, another study showed that KDML105 rice grains had a slightly higher 2AP content when grown in clay loam ( $3.32 \text{ mg kg}^{-1}$ ) than in sandy loam ( $2.87 \text{ mg kg}^{-1}$ ) [49]. In the present study, on average, finer textured soils tended to increase the 2AP content of rice grains more than coarser textured soils did (Figure 9). It is interesting to note that the farming practice exerted a stronger influence on the 2AP content of the rice grains than on the soil texture. Under the same soil texture, the 2AP level obtained from the ORF system was much higher than that obtained from the CRF system (Figure 9).

Owing to the low natural fertility of the TKR soils that exhibit high spatial variability, attempts have been made to improve rice crop yield by various soil management methods. Chemical fertilizer addition in the TKR region has not been very successful as this practice exerts a negative effect on the aromatic content of KDML105 [49]. Recently, much attention has been paid to improving the TKR soil fertility, rice yield, and quality with organic matter (OM) application. The results of the present study indicated that the organic fertilizer application in the ORF system not only increased the SOM level but also resulted in much higher TN

and 2AP values compared to the values obtained from the CRF system (Figures 10(a) and 10(b)). The higher TN in the ORF system (Figure 10(b)) may be one of the main causal agents of a much higher 2AP level in this system because 2AP is an N-containing aromatic compound. Yang et al. [10] concluded that TN in the soil is one of the key factors in the aroma production of Chinese aromatic rice.

The higher HA content in the ORF system may have also contributed to higher 2AP content in the rice grains than in the CRF system (Figure 10(c)). HA derived from organic matter decomposition contains many types of N compounds, including polyamines. Although proline is known as the precursor for the biosynthesis of 2AP [50], a study on the aromatic gene *Os2AP* in KDML rice seedlings indicated that 2AP is synthesized via the polyamine pathway [51]. Therefore, we suggested that organic N, including polyamines in HA, may also play an important role in 2AP increments of the ORF system in TKR. Pearson correlation coefficients were calculated among the 2AP concentrations, soil properties, and microbial populations (Table 2). A significantly positive correlation at  $p < 0.01$  was found between SOM ( $0.8858^{***}$ ), HA ( $0.6881^{***}$ ), and TN



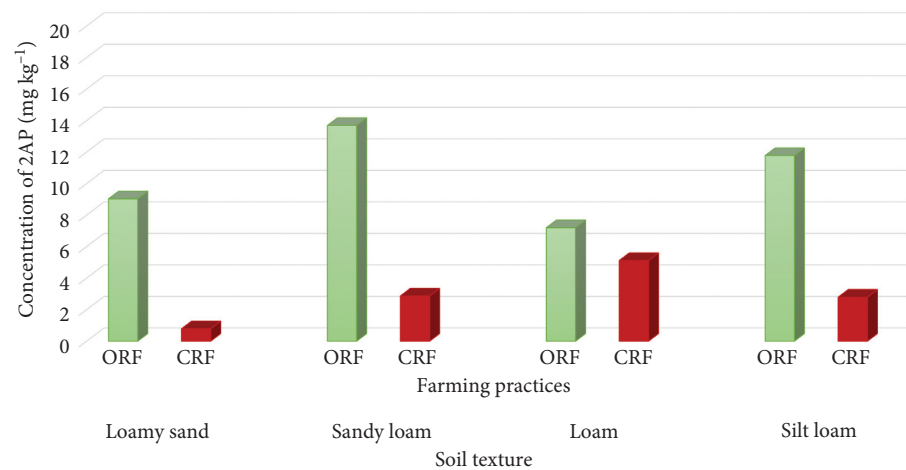


FIGURE 9: The 2-acetyl-1-pyrroline (2AP) content in Khao Dawk Mali 105 (KDML105) grains as affected by soil texture groups. Note: ORF= organic rice farming; CRF = conventional rice farming.

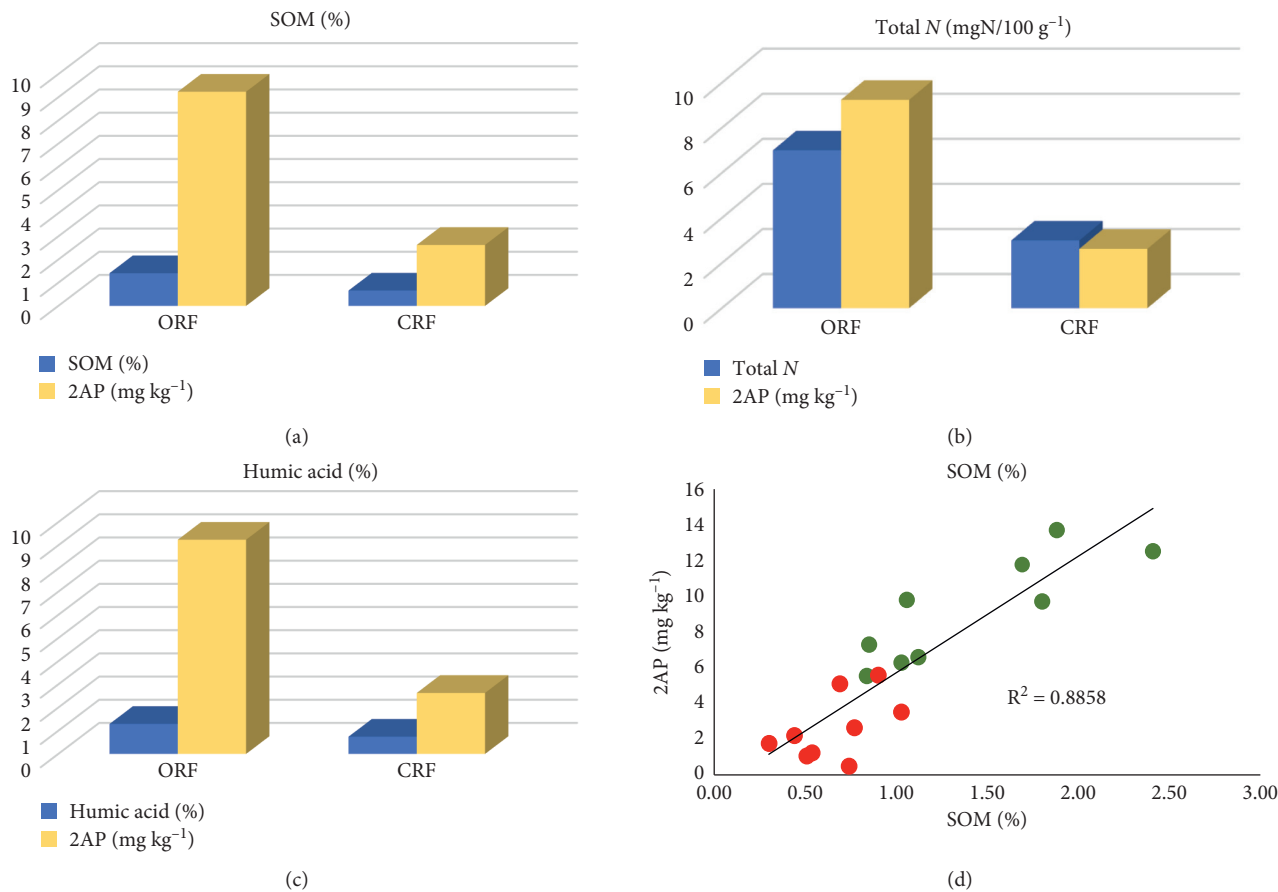


FIGURE 10: Continued.

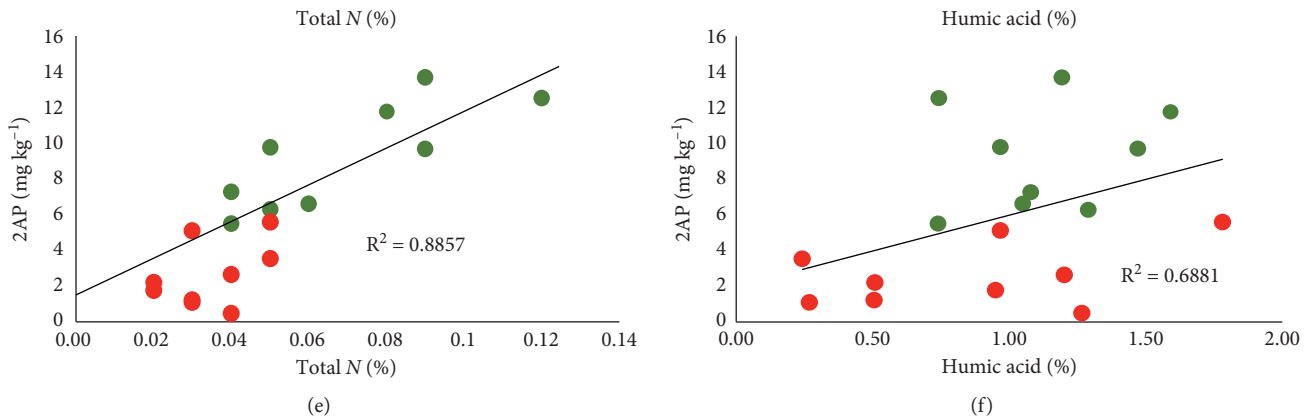


FIGURE 10: The amount of 2-acetyl-1-pyrroline (2AP) content in Khao Dawk Mali 105 (KDML105) rice grains as affected by soil organic matter (SOM) (a), total nitrogen (TN) (b), and humic acid (HA) (c) and their relationship with 2AP (d e, f). Note: ORF = organic rice farming; CRF = conventional rice farming.

TABLE 2: Pearson's correlation matrix for the 2-acetyl-1-pyrroline (2AP) concentrations, physicochemical properties, and soil microbial populations.

<i>p</i> values	2AP	pH	OM	Humic acid	EC	CEC	Total N	Bacteria	Actinomycetes	Fungi
<i>r</i> values										
2AP										
pH	−0.4333									
OM	<b>0.8858***</b>	−0.2238								
Humic acid	<b>0.6881***</b>	−0.2612	<b>0.5402**</b>							
EC	<b>0.5259***</b>	−0.234	0.3568	0.4115						
CEC	0.1946	−0.6121	0.0652	0.0936	0.3822					
Total N	<b>0.8857***</b>	−0.2238	1	<b>0.5402**</b>	0.3568	0.0652				
Bacteria	<b>0.7646***</b>	−0.341	<b>0.6269***</b>	0.3964	<b>0.5007**</b>	0.2447	<b>0.6269***</b>			
Actinomycetes	<b>0.8364***</b>	−0.2159	<b>0.7768***</b>	<b>0.6595***</b>	<b>0.5958***</b>	0.1334	<b>0.7768***</b>	0.4837		
Fungi	<b>0.7509***</b>	−0.3731	<b>0.793***</b>	<b>0.5179**</b>	<b>0.5069***</b>	0.0542	<b>0.7930***</b>	<b>0.6334***</b>	<b>0.6518***</b>	

\*\*, \*\*\*significant correlational  $p < 0.05$  and  $0.01$ , respectively.

(0.8857\*\*\*)) and the 2AP concentration (Table 2; Figures 10(d)–10(f)).

**3.2.3. 2AP Affected by Soil Microbial Populations.** The organic inputs in the ORF system in this study not only increased the SOM, TN, and HA values but also the microbial population of KDML105. These soil factors showed a high positive correlation with 2AP levels in KDML105 grains (Figures 10 and 11). A significantly positive correlation ( $p < 0.01$ ) was found between the bacterial (0.7646\*\*\*), actinomycetal (0.8364\*\*\*), and fungal (0.7509\*\*\*)) populations and the 2AP concentration (Figure 11; Table 2). These results indicated the high impact of rice rhizosphere microorganisms on the 2AP level in KDML 105 rice grains. It was reported that several bacterial genera such as *Bacillus*, *Acinetobacter*, *Pseudomonas*, and *Enterobacter* increased the 2AP level of aromatic rice by 1.14–1.42-fold [52]. Therefore, it could be concluded from this study that the higher microbial population in the ORF system might be one of the key factors in enhancing 2AP synthesis in the rice grains.

**3.3. Principal Component Analysis for the 2AP Content Concentrations and Soil Properties.** PCA provides good information on the relationship among variables. The relationship between the farming practices, soil properties, and rhizosphere microorganisms and the 2AP examined by PCA allowed us to characterize each horizon type (Figure 12). The first two components explained 60.7% of the total variability; component 1 explained 35.1%, while component 2 explained 25.6%. Organic and conventional farming were identified on the correlation circle. It appeared that most of the soil properties showed a positive relationship with the ORF system. The results of the PCA clearly indicated that the SOM, TN, HA, EC, bacterial, actinomycetal, and fungal values had the strongest correlation with 2AP in the rice grains. A very close relationship was also found among SOM, TN, HA, and the microbial population. A very high correlation between these soil factors and 2AP appeared to contribute to the ORF practices and was explained by the fact that high SOM in the ORF soil is a source of N, carbon, and energy for microorganisms and that HA is synthesized by their activity (Figure 12). In addition, 2AP is an N-containing volatile compound, and its synthesis can be

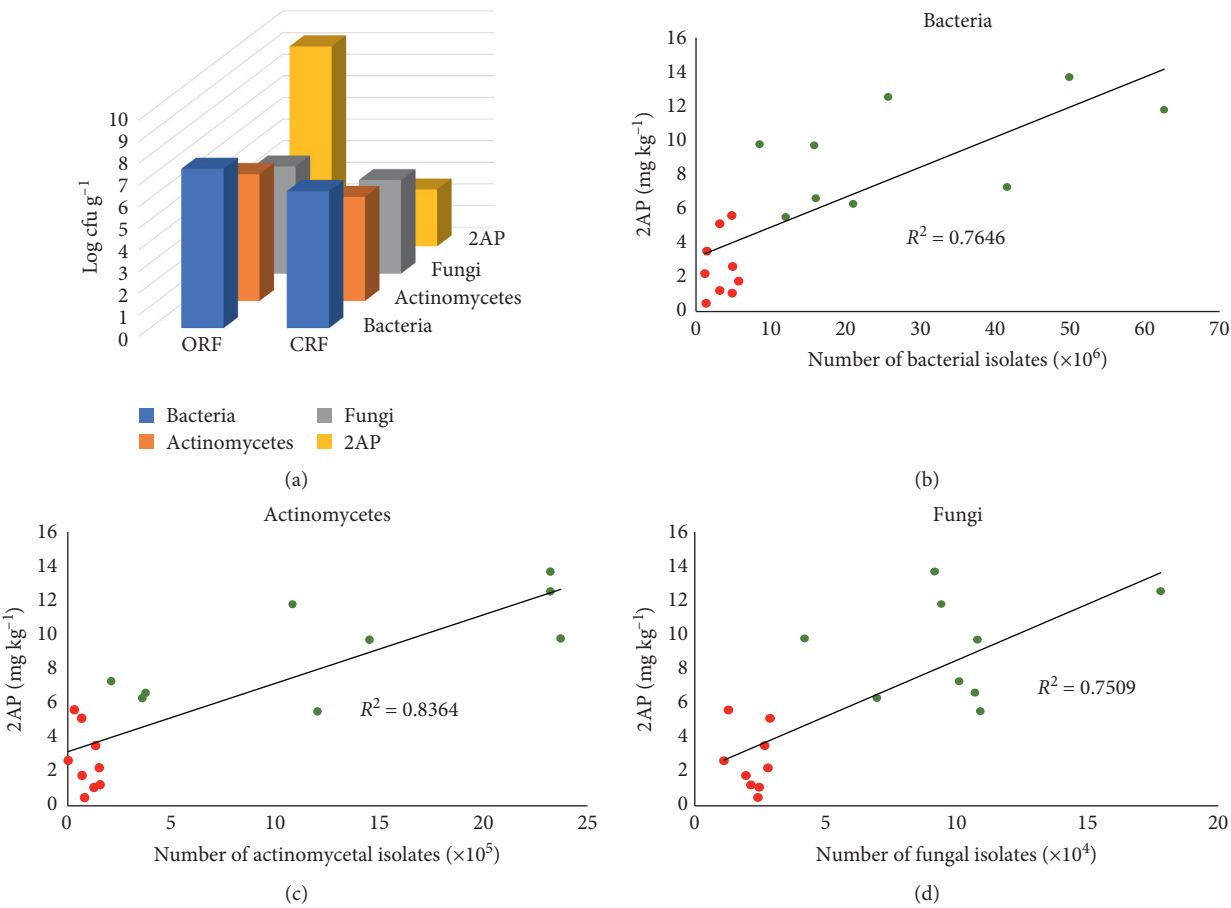


FIGURE 11: Influence of soil microbial populations on the 2-acetyl-1-pyrroline (2AP) content in Khao Dawk Mali 105 (KDML105) rice grains (a) and their relationship with the 2AP content (b), (c), and (d). Note: ORF = organic rice farming; CRF = conventional rice farming.

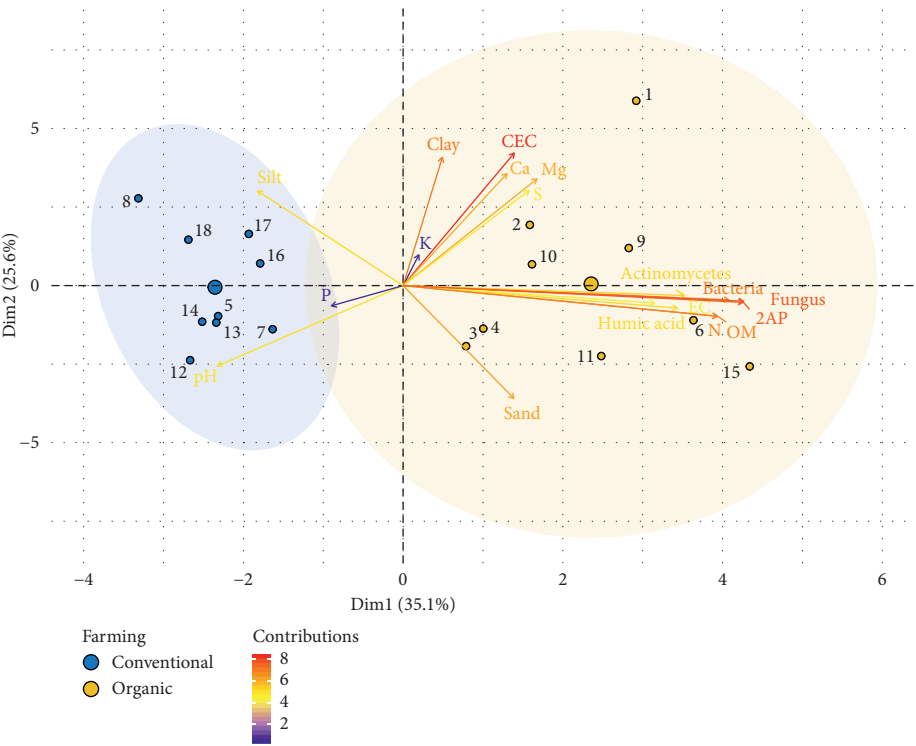


FIGURE 12: Principal component analysis for cultivation practices, soil properties, soil microbial population, and 2-acetyl-1-pyrroline (2AP).

enhanced by rhizosphere microorganisms. Other variables appeared to have much less or no correlation with 2AP.

#### 4. Conclusions

Organic rice farming (ORF) is markedly better in enhancing soil microbial population and major soil properties, particularly soil organic matter (SOM), than conventional rice farming (CRF). The higher soil quality in the ORF had a strong positive impact on the 2AP content in KDML105 rice grains. Our results highlighted the key role of SOM in improving soil quality and its potential to increase the potential of KDML105 in 2AP synthesis, thereby also increasing its environmental stress tolerance in the TKR region. Therefore, the ORF system is highly recommended in the TKR region for the improvement of soil properties and rice grain quality, particularly in terms of 2AP production. However, more field research in the TKR region is required to better characterize the complex interactions among soil factors and their influences on rice yield and the 2AP content of KDML105.

#### Data Availability

The data used to support the findings of this study are available from the first or corresponding author upon request (the data in this research paper are a part of Kawiporn's PhD thesis, and all the contents will be copyrighted by Chiang Mai University).

#### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

#### Acknowledgments

The authors are indebted to the Graduate School, Chiang Mai University, for the TA/RA scholarship support and wish to thank the farmers of the Community Enterprise of Thamo Organic Agricultural Group, Surin Province, and Na So Rice Farmers Group (Nature Conservation Club), Kut Chum, Yasothon Province, for their help during field sampling.

#### References

- [1] V. D. Daygon, S. Prakash, M. Calingacion et al., "Understanding the jasmine phenotype of rice through metabolite profiling and sensory evaluation," *Metabolomics*, vol. 12, no. 4, pp. 1–15, 2016.
- [2] A. Gaur, H. S. Wani, D. Pandita, N. Bharti, and A. Malav, "Understanding the fragrance in rice," *Journal Rice Research*, vol. 4, no. 1, p. 125, 2016.
- [3] Z. A. Jewel, A. K. Patwary, S. Maniruzzaman, R. Barua, and S. N. Begum, "Physico-chemical and genetic analysis of aromatic rice (*Oryza sativa* L.) Germplasm," *The Agriculturists*, vol. 9, no. 1–2, pp. 82–88, 2011.
- [4] F. S. G. Hashemi, M. Y. Rafii, M. R. Ismail et al., "Biochemical, genetic and molecular advances of fragrance characteristics in rice," *Critical Reviews in Plant Sciences*, vol. 32, no. 6, pp. 445–457, 2013.
- [5] H. S. Ko, T. H. Kim, J.-Y. Yang, Y.-S. Kim, and H. J. Lee, "Aroma active compounds of bulgogi," *Journal of Food Science*, vol. 70, no. 8, pp. c517–c522, 2005.
- [6] M. Yi, K. T. Nwe, A. Vanavichit, W. Chai-arree, and T. Toojinda, "Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthu-kha," *Field Crops Research*, vol. 113, no. 2, pp. 178–186, 2009.
- [7] P. Goufo, M. Duan, S. Wongpornchai, and X. Tang, "Some factors affecting the concentration of the aroma compound 2-acetyl-1-pyrroline in two fragrant rice cultivars grown in South China," *Frontiers of Agriculture in China*, vol. 4, no. 1, pp. 1–9, 2010.
- [8] S. Panichapong and S. Distribution, "Characteristics and utilization of problem soils in Thailand," *Tropical Agriculture Research Series*, vol. 15, pp. 83–96, 1982.
- [9] T. Yoshihashi, "Quantitative analysis on 2-acetyl-1-pyrroline of an aromatic rice by stable isotope dilution method and model studies on its formation during cooking," *Journal of Food Science*, vol. 67, no. 2, pp. 619–622, 2002.
- [10] S. Yang, Y. Zou, Y. Liang et al., "Role of soil total nitrogen in aroma synthesis of traditional regional aromatic rice in China," *Field Crops Research*, vol. 125, pp. 151–160, 2012.
- [11] Z. Mo, J. Huang, D. Xiao et al., "Supplementation of 2-Ap, Zn and La improves 2-acetyl-1-pyrroline concentrations in detached aromatic rice panicles in vitro," *PLoS One*, vol. 11, no. 2, Article ID e0149523, 2016.
- [12] R. P. Day, "Pipette method of particle size analysis," in *Methods of Soil Analysis: Agronomy*, vol. 9, pp. 553–562, American Society of Anesthesiologists, USA, 1965.
- [13] D. Eckert and J. T. Sims, "Recommended Soil pH and Lime Requirement Tests," *Recommended Soil Testing Procedures for the Northeastern United States*, Northeastern Regional Publication, vol. 493, no. 19–26, USA, 3rd edition, 1995.
- [14] H. D. Chapman, "Cation exchange capacity," in *Method of Soil Analysis—Part 2*, C. A. Black, D. D. Evans, L. E. Ensminger, J. L. White, F. E. Clark, and R. C. Dinauer, Eds., pp. 891–913, American Society of Agronomy, Madison, WI, USA, 1965.
- [15] J. M. Bremner, "Total nitrogen," in *Methods of Soil Analysis—Part 2*, C. A. Black, D. D. Evans, L. E. Ensminger et al., Eds., pp. 914–926, American Society of Agronomy, Madison, WI, USA, 1965.
- [16] O. H. Ahmed, M. H. A. Husni, A. R. Anuar, and M. M. Hanafi, "Effects of extraction and fractionation time on the yield of compost humic acids," *New Zealand Journal of Crop and Horticultural Science*, vol. 33, no. 2, pp. 107–110, 2005.
- [17] P. Palanivell, K. Susilawati, O. H. Ahmed, and N. M. Majid, "Compost and crude humic substances produced from selected wastes and their effects on Zea mays L. Nutrient uptake and growth," *Corporation The Scientific World Journal*, vol. 2013, Article ID 276235, 2013.
- [18] E. E. Schulte and B. Hoskin, "Recommended soil organic matter tests," in *Recommended Soil Testing Procedures for the Northeastern United States*, R. Rhodes, Ed., vol. 493, pp. 52–60, Northeastern Regional Publication, USA, 3rd edition, 2011.
- [19] R. H. Bray and L. T. Kurtz, "Determination of total, organic, and available forms of phosphorus in soils," *Soil Science*, vol. 59, no. 1, pp. 39–46, 1945.
- [20] P. R. Hesse, "The effect of colloidal organic matter on the precipitation of barium sulphate and a modified method for determining soluble sulphate in soils," *Analyst*, vol. 82, pp. 710–712, 1957.
- [21] A. Wolf and D. Beegle, "Recommended soil tests for macronutrients: phosphorus, potassium, calcium, and

- magnesium,” in *Recommended Soil Testing Procedures for the Northeastern United States*, pp. 30–38, Northeastern regional Publication No. 453, Newark, DE, USA, 2nd edition, 1995.
- [22] K. Alef, “Enrichment, isolation and counting of soil microorganisms,” in *Methods in Applied Soil Microbiology and Biochemistry*, K. Alef and P. Nannipieri, Eds., pp. 123–192, Academic Press, San Diego, CA, USA, 1995.
- [23] S. Wongpornchai, T. Sriseadka, and S. Choonvisase, “Identification and quantitation of the rice aroma compound, 2-Acetyl-1-pyrroline, in bread flowers (*Vallisneria spiralis* L.),” *Journal of Agricultural and Food Chemistry*, vol. 51, no. 2, pp. 457–462, 2003.
- [24] R Development Core Team, *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing, Vienna, Austria, 2011.
- [25] P. Duangpatra, “Soil and climate characterization of major cassava growing areas in Thailand,” in *Cassava breeding and agronomy research in Asia*, in *Proceedings of the A Regional Workshop Held in Rayong*, R. H. Howeler and K. Kawano, Eds., Thailand, October 1998.
- [26] S. Marinari, R. Mancinelli, E. Campiglia, and S. Grego, “Chemical and biological indicators of soil quality in organic and conventional farming systems in Central Italy,” *Ecological Indicators*, vol. 6, no. 4, pp. 701–711, 2006.
- [27] C. Quantin, O. Grunberger, N. Suvannang, and E. Bourdon, “Impact of agricultural practices on the biogeochemical functioning of sandy salt-affected paddy soils in Northeastern Thailand,” in *Proceedings of the First Symposium on the Management of Tropical Sandy Soils for Sustainable Agriculture*, Khon Kaen, Thailand, 2005.
- [28] B. Minasny, S. Y. Hong, A. E. Hartemink, Y. H. Kim, and S. S. Kang, “Soil pH increase under paddy in South Korea between 2000 and 2012,” *Agriculture, Ecosystems & Environment*, vol. 221, pp. 205–213, 2016.
- [29] E. Ozlu and S. Kumar, “Response of soil organic carbon, pH, electrical conductivity, and water stable aggregates to long-term annual manure and inorganic fertilizer,” *Soil Science Society of America Journal*, vol. 82, no. 5, pp. 1243–1251, 2018.
- [30] Z. Demir and C. Gülser, “Effects of rice husk compost application on soil quality parameters in greenhouse conditions,” *Eurasian Journal of Soil Science (EJSS)*, vol. 4, no. 3, pp. 185–190, 2015.
- [31] R. A. Eigenberg, J. W. Doran, J. A. Nienaber, R. B. Ferguson, and B. L. Woodbury, “Electrical conductivity monitoring of soil condition and available N with animal manure and a cover crop,” *Agriculture, Ecosystems & Environment*, vol. 88, no. 2, pp. 183–193, 2002.
- [32] F. Candemir and C. Gülser, “Effects of different agricultural wastes on some soil quality indexes in clay and loamy sand fields,” *Communications in Soil Science and Plant Analysis*, vol. 42, no. 1, pp. 13–28, 2011.
- [33] N. Arunrat, N. Pumijumong, and R. Hatano, “Practices sustaining soil organic matter and rice yield in a tropical monsoon region,” *Soil Science and Plant Nutrition*, vol. 63, no. 3, pp. 274–287, 2017.
- [34] S. Wang, X. Wang, and Z. Ouyang, “Effects of land use, climate, topography and soil properties on regional soil organic carbon and total nitrogen in the Upstream Watershed of Miyun Reservoir, North China,” *Journal of Environmental Sciences*, vol. 24, no. 3, pp. 387–395, 2012.
- [35] X. Hao and A. N. Kravchenko, “Management practice effects on surface soil total carbon: differences along a textural gradient,” *Agronomy Journal*, vol. 99, no. 1, pp. 18–26, 2007.
- [36] P. Boontakham, P. Sookwong, P. Sookwong, S. Jongkaewwattana, S. Wangtueai, and S. Mahatheerant, “Comparison of grain yield and 2-acetyl-1-pyrroline (2AP) content in leaves and grain of two Thai fragrant rice cultivars cultivated at greenhouse and open-air conditions,” *Australian Journal of Crop Science*, vol. 13, no. 1, pp. 159–169, 2019.
- [37] J. Zhang, J. Wang, T. An et al., “Effects of long-term fertilization on soil humic acid composition and structure in black soil,” *PLoS One*, vol. 12, no. 11, Article ID e0186918, 2017.
- [38] S. Nardi, A. Muscolo, S. Vaccaro, S. Baiano, R. Spaccini, and A. Piccolo, “Relationship between molecular characteristics of soil humic fractions and glycolytic pathway and krebs cycle in maize seedlings,” *Soil Biology and Biochemistry*, vol. 39, no. 12, pp. 3138–3146, 2007.
- [39] W. Mindari, P. E. Sasongko, Z. Kusuma, Syekhfan, and N. Aini, “Efficiency of various sources and doses of humic acid on physical and chemical properties of saline soil and growth and yield of rice,” *AIP Conference Proceedings*, vol. 2019, no. 030001, 2018.
- [40] W. Saetung and V. Trelo-ge, “Monitoring in soil fertility change in Tung Kula Rong Hai using geographic information systems,” *International Research Journal of Advanced Engineering and Science*, vol. 2, no. 4, pp. 189–193, 2017.
- [41] R. L. Parfitt, D. J. Giltrap, and J. S. Whitton, “Contribution of organic matter and clay minerals to the cation exchange capacity of soils,” *Communications in Soil Science and Plant Analysis*, vol. 26, no. 9–10, pp. 1343–1355, 1995.
- [42] C. Hu, X. Xia, Y. Chen, and X. Han, “Soil carbon and nitrogen sequestration and crop growth as influenced by long-term application of effective microorganism compost,” *Chilean Journal of Agricultural Research*, vol. 78, no. 1, pp. 13–22, 2018.
- [43] A. Araújo, L. Leite, V. Santos, and R. Carneiro, “Soil microbial activity in conventional and organic agricultural systems,” *Sustainability*, vol. 1, no. 2, pp. 268–276, 2009.
- [44] W. Wang, Y. Li, P. Dang et al., “Rice secondary metabolites: structures, roles, biosynthesis, and metabolic regulation,” *Molecules*, vol. 23, no. 12, pp. 1–50, 2018.
- [45] S. Saha, A. K. Pandey, K. A. Gopinath, R. Bhattacharaya, S. Kundu, and H. S. Gupta, “Nutritional quality of organic rice grown on organic composts,” *Agronomy for Sustainable Development*, vol. 27, no. 3, pp. 223–229, 2007.
- [46] K. Surekha, K. V. Rao, R. N. Shobha, P. C. Latha, and R. M. Kumar, “Evaluation of organic and conventional rice production systems for their productivity, profitability, grain quality and soil health,” *Agrotechnology*, vol. 1, no. S11, p. 6, 2013.
- [47] P. Poomipan, S. Chakatrakan, V. Latkanathinnawong, C. Pliumchareorn, and P. Chomphuphiw, “Comparison between chemical fertilizer and high quality organic fertilizer on quality of rice variety suphan buri 1,” *Thai Journal of Science and Technology*, vol. 24, no. 5, pp. 753–765, 2016.
- [48] K. Pisithkul, S. Jongkaewwattana, S. Mahatheerant, V. Tulyathan, and S. Meechoui, “Effect of accelerated aging treatments on aroma quality and major volatile components of Thai jasmine rice,” *Chiang Mai University Journal of Natural Sciences*, vol. 9, no. 2, pp. 281–294, 2010.
- [49] A. Suwannarit, S. Buranakarn, S. Kritapirom et al., “The relationship between trace element fertilizer, sulfur, sodium, salinity of the soil and harvesting with yield and quality cooking of white rice,” *Jasmine*, vol. 105, 2012.
- [50] C. C. Young and L. F. Chen, “Polyamines in humic acid and their effect on radical growth of lettuce seedlings,” *Plant and Soil*, vol. 195, no. 1, pp. 143–149, 1997.



- [51] A. Vanavichit, T. Yoshihashi, S. Wanchana et al., "Cloning of *os2ap*, the aromatic gene controlling the biosynthetic switch of 2-acetyl-1-pyrroline and gamma aminobutyric acid (GABA) in rice," in *Proceedings of the 5th International Rice Genetics Symposium*, vol. 44, pp. 19–23, Manila, Philippines, November 2005.
- [52] Y. Deshmukh, P. Khare, and D. Patra, "Rhizobacteria elevate principal basmati aroma compound accumulation in rice variety," *Rhizosphere*, vol. 1, pp. 53–57, 2016.